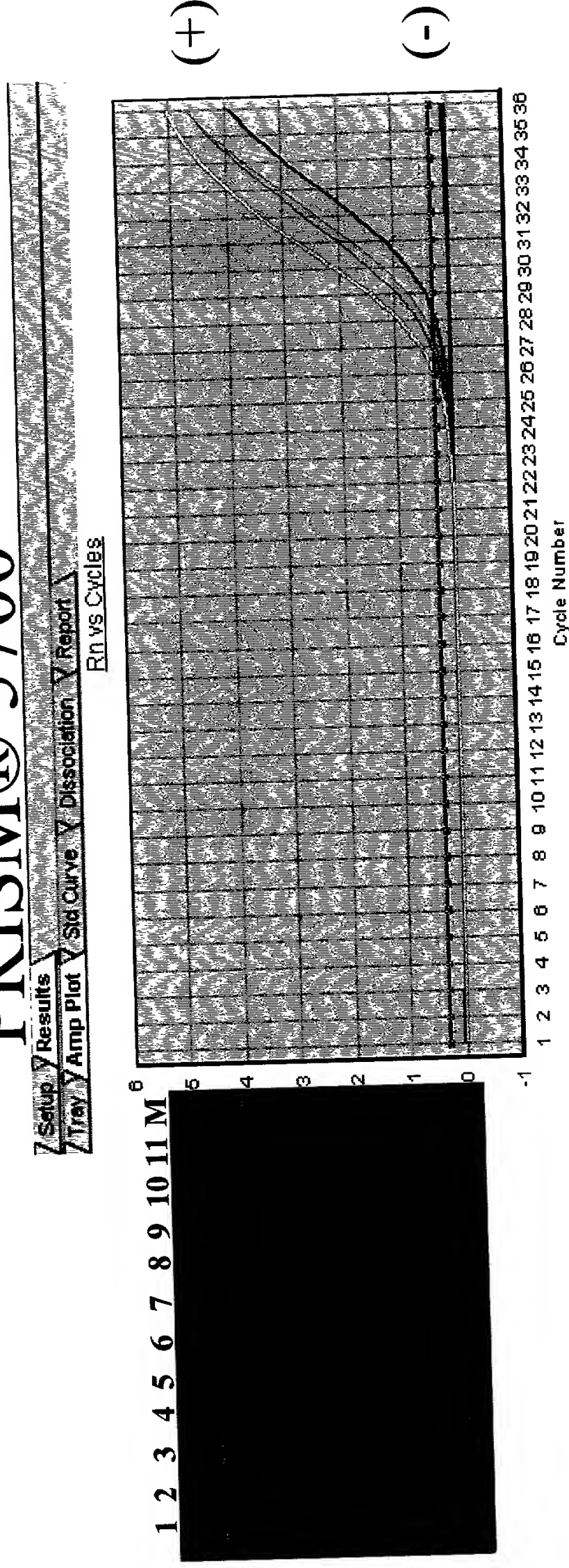# Comparison of Agarose Gel with ABI PRISM® 5700

Figure 1. The agarose gel and the ABI PRISM® 5700 show different methods of evaluating PCR results. The gel shows the three positive PCR reactions (Lanes 1, 3 & 9), as well as a control ladder (Lane 12). The agarose gel also shows eight negative PCR reactions. The ABI PRISM® 5700 Sequence Detection System generates an Amplification Plot, which is a measurement of the increase in fluorescence of SYBR green. This increase correlates to an increase of PCR products. The above Amplification Plot shows the four positive reactions (+), and the eight negative reactions (-). The data in the Amplification Plot was collected during the PCR amplification, and the analyzed data was available immediately upon completion of the PCR reactions. The gel shows three positive reactions and not four because the positive control was not loaded, and the control ladder was run in its place.

Low Resolution Typing
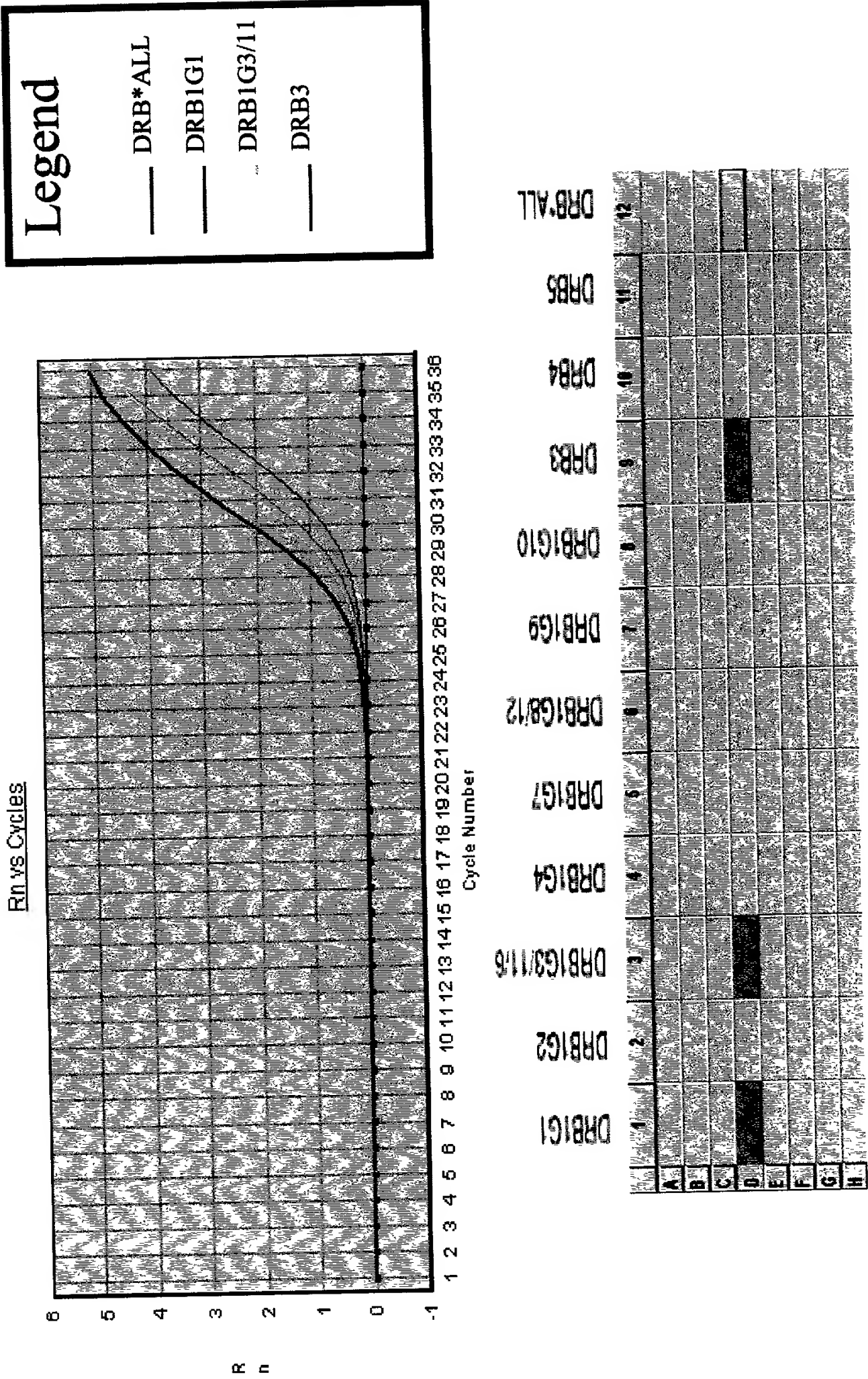
Figure 2. Based on the PCR results, this person is positive for DRB1G1, DRB1G3/11/6 and DRB3. This is an expected combination. This completes the low resolution typing of this individual. These same PCR products were then used for high resolution typing.

High Resolution Typing
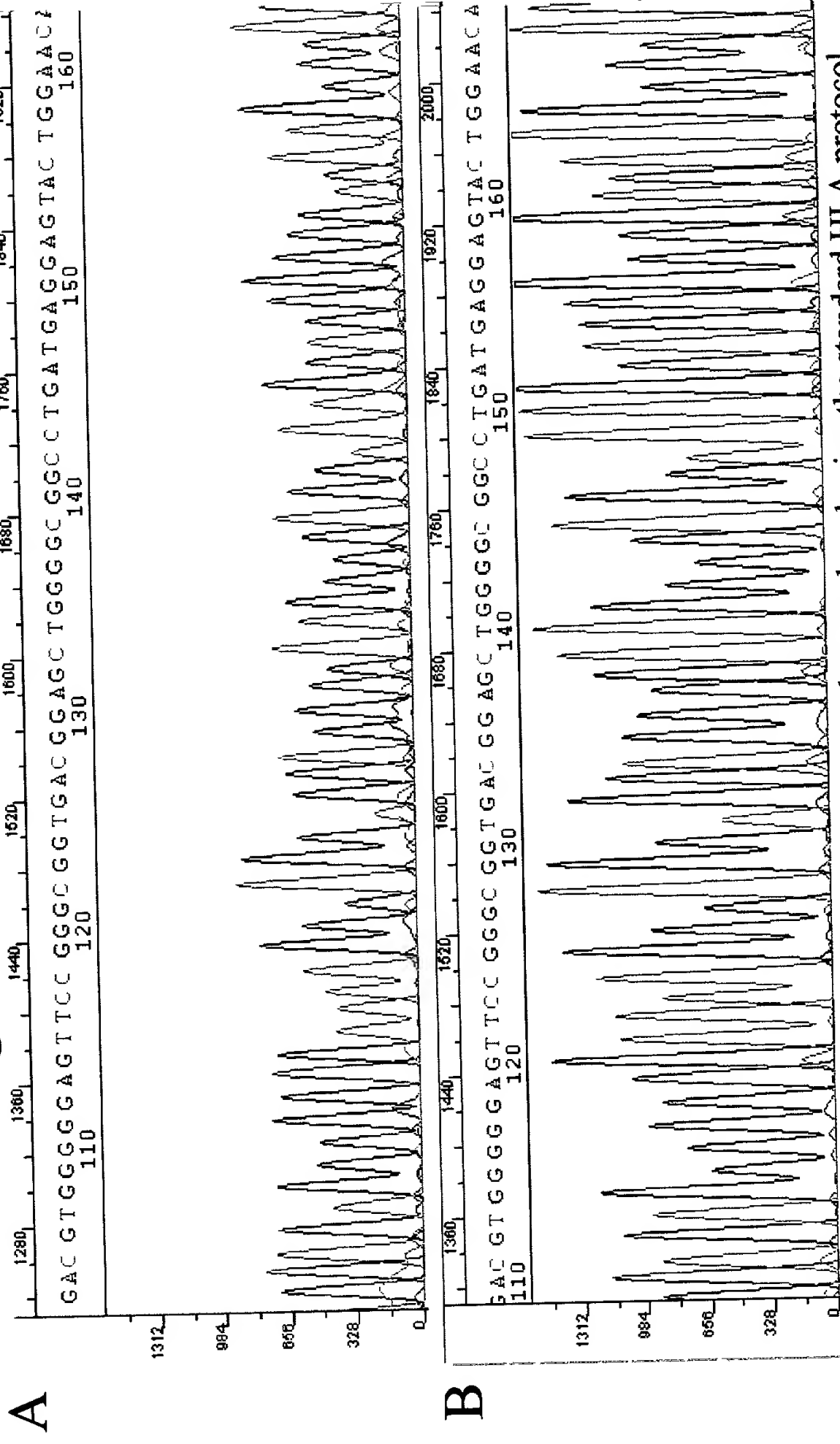
Figure 3. Panel A, shows sequence data from a PCR product produced using the standard HLA protocol. Panel B, shows sequence data from a PCR product generated using the modified SYBR/HLA protocol. Each sample was immediately sequenced after low resolution typing was completed. This comparison of data shows, the addition of SYBR® Green PCR Master Mix had no adverse effect on the sequencing reaction. This data was produced on the ABI PRISM® 3100 Genetic Analyzer.

DF Preliminary Report

Sample: **DF3**   Library: **DRB1.L155**
Preliminary Report: Exact match to **11011 11011/1105 1105.**   See Warning

Files DF3IF.ab1, DF3IR.ab1

Warning #3: There are 1 ambiguities at polymorphic positions

Warning #6: There are unexpected base calls at these 1 constant positions

```
1 | nucleotide
0 |   number
1 |
```

```
A    DRB1.L155 consensus
M >  DF3IF.ab1
A <  DF3IR.ab1
```

Warnings for file DF3IF.ab1
#9: The sequence was analyzed with the wrong version of Sequencing Analysis
#10: The model 3100 sequencer used is not valid.
#11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to

Warnings for file DF3IR.ab1
#9: The sequence was analyzed with the wrong version of Sequencing Analysis
#10: The model 3100 sequencer used is not valid.
#11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to

Polymorphic Position Report

```
       11111 111111111 1111222222 2222222222 22222222 | nucleotide
4444577788 8899900011 1114567777 7779000111 1111222333 33555567 |   number
0268747824 89147285
GGCTTGTCGC TATTAAGTA
```

```
                                                                     10
  1 DF1[F.ab1    ACGGAGCGGG TGCGGTTCGT GGAAAAGATGC
  5
  2E DF1[R.ab1   ACGGAGCGGG TGCGGTTCCT GGAAAAGATGC
  6
  3 DF3[F.ab1    ACGGAGCGGG TGCGGTTCCT GGACAGATAC
  6
  4E DF3[R.ab1   ACGGAGCGGG TGCGGTTCCT GGACAGATAC
        70              80              0
```

DF Final Report

Sample: **DF3**   Library: **DRB1.L155**
Allele Report: Exact match to **11011 11011/1105 1105.**   See Warnings Bel

Files . DF3IF.ab1, DF3IR.ab1

Warning #3: There are 1 ambiguities at polymorphic positions.

Warnings for file: DF3IF.ab1
#9: The sequence was analyzed with the wrong version of Sequencing Analysis
#10: The model 3100 sequencer used is not valid.
#11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to

Warnings for file: DF3IR.ab1
#9: The sequence was analyzed with the wrong version of Sequencing Analysis
#10: The model 3100 sequencer used is not valid.
#11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to

Polymorphic Position Report

```
       11111 111111111 1111222222 2222222222 22222222 | nucleotide
4444577788 8899900011 1114567777 7779000111 1111222333 33555557 |   number
0268747824 89147285902 3790990134 5899789012 4568012012 342478490 |
GGCTTGTCGC TATTAAGTAG TCTTGGAT*G GTATAGACAG CGGCGCGCCT ACGTGTCA <>   DF3 cons
                                                    R                   ^    DF3IF.db
                                                    M                   v    DF3IR.db
```

```
                                                                     11011
                                                                     11011/1105
                                                                     1105
                                                                R    11011/11012
                                                                     130
  G AATGTCATTT CTTCAATGGG
  G AATGTCATTT CTTCAATGGG
    AGTGTCATTT CTTCAATGGG
  G AGTGTCATTT CTTCAATGGG
        110             120
                  ATCTTATAACC AAGAGGAGTC CGTGCGCTTC GACAGCGACG
                  ATCTTATAACC AAGAGGAGTC CGTGCGCTTC GACAGCGACG
                  TTCTATAACC  aAGAGGAGTA CGTGCGCTTC GACAGCGACG
                  TTCTATAACC  AAGAGGAGTA CGTGCGCTTC GACAGCGACG
        90              100
```

Figure 4. This panel shows the completion of the high resolution typing. The sequenced sample data was analyzed by the Applied Biosystems MatchTools™ software to get a Preliminary Report. The data was then edited in Applied Biosystems MT Navigator software, before being resubmitted to the Applied Biosystems MatchTools™ software for a Final Report. This sample was an exact match to 11011, 11011/1105, 1105.